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Bisphosphonates: From the Laboratory to the Clinic and Back Again

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Bisphosphonates (BPs) used as inhibitors of bone resorption all contain two phosphonate groups attached to a single carbon atom, forming a "P-C-P" structure. The bisphosphonates are therefore stable analogues of naturally occuring pyrophosphate-containing compounds, which now helps to explain their intracellular as well as their extracellular modes of action. Bisphosphonates adsorb to bone mineral and inhibit bone resorption. The mode of action of bisphosphonates was originally ascribed to physico-chemical effects on hydroxyapatite crystals, but it has gradually become clear that cellular effects must also be involved. The marked structure-activity relationships observed among more complex compounds indicate that the pharmacophore required for maximal activity not only depends upon the bisphosphonate moiety but also on key features, e.g., nitrogen substitution in alkyl or heterocyclic side chains.

Several bisphosphonates (e.g., etidronate, clodronate, pamidronate, alendronate, tiludronate, risedronate, and ibandronate) are established as effective treatments in clinical disorders such as Paget's disease of bone, myeloma, and bone metastases. Bisphosphonates are also now well established as successful antiresorptive agents for the prevention and treatment of osteoporosis. In particular, etidronate and alendronate are approved as therapies in many countries, and both can increase bone mass and produce a reduction in fracture rates to approximately half of control rates at the spine, hip, and other sites in postmenopausal women. In addition to inhibition of osteoclasts, the ability of bisphosphonates to reduce the activation frequency and birth rates of new bone remodeling units, and possibly to enhance osteon mineralisation, may also contribute to the reduction in fractures.

The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption, but highly selective localization and retention in bone. Significant side effects are minimal. Current issues with bisphosphonates include the introduction of new compounds, the choice of therapeutic regimen (e.g., the use of intermittent dosing rather than continuous), intravenous vs. oral therapy, the optimal duration of therapy, the combination with other drugs, and extension of their use to other conditions, including steroid-associated osteoporosis, male osteoporosis, arthritis, and osteopenic disorders in childhood.

Bisphosphonates inhibit bone resorption by being selectively taken up and adsorbed to mineral surfaces in bone, where they interfere with the action of osteoclasts. It is likely that bisphosphonates are internalized by osteoclasts and interfere with specific biochemical processes and induce apoptosis. The molecular mechanisms by which these effects are brought about are becoming clearer. Recent studies show that bisphosphonates can be classified into at least two groups with different modes of action. Bisphosphonates that closely resemble pyrophosphate (such as clodronate and etidronate) can be metabolically incorporated into nonhydrolysable analogues of ATP that may inhibit ATP-dependent intracellular enzymes. The more potent, nitrogen-containing bisphosphonates (such as pamidronate, alendronate, risedronate, and ibandronate) are not metabolized in this way but can inhibit enzymes of the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small GTPases. The inhibition of protein prenylation and the disruption of the function of these key regulatory proteins explains the loss of osteoclast activity and induction of apoptosis. These different modes of action might account for subtle differences between compounds in terms of their clinical effects.

In conclusion, bisphosphonates are now established as an important class of drugs for the treatment of bone diseases, and their mode of action is being unravelled. As a result, their full therapeutic potential is gradually being realized. (Bone 25:97–106; 1999)

Key Words: Bone resorption; Osteoclast; Bisphosphonates; Protein prenylation; Bone Metastases; Myeloma; Osteoporosis.

Introduction

The discovery and development of the bisphosphonates (BPs) as a major class of drugs for the treatment of bone diseases has been a fascinating story that has extended over three decades. As the title of this presentation implies, the tale starts with laboratory studies[31,32] related to mechanisms of biological calcification, followed by the clinical exploitation of bisphosphonates as inhibitors of bone resorption, and has recently returned to laboratory studies that are helping to unravel how these drugs work at a cellular level.

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There are several recent books and reviews available that describe the chemistry, pharmacology, and clinical applications of bisphosphonates.[10,22,27,28,37,41,43,47,73,79,93]

The Early Days

In the early 1960s, Neuman and Fleisch[30] were studying mechanisms of calcification induced by collagen, and showed that body fluids such as plasma and urine contained inhibitors of calcification. Since it had been known since the 1930s that trace amounts of polyphosphates were capable of acting as water softeners by inhibiting the crystallization of calcium salts, such as calcium carbonate, they proposed that compounds of this type might be natural regulators of calcification under physiological conditions.[27] Fleisch and his colleagues showed that inorganic pyrophosphate, a naturally occurring polyphosphate and a known by-product of many biosynthetic reactions in the body, was present in serum and urine and could prevent calcification by binding to newly forming crystals of hydroxyapatite.[29,34] It was therefore postulated that pyrophosphate (PPi) might be the agent that normally prevents calcification of soft tissues, and regulates bone mineralization. Pathological disorders, such as the formation of kidney stones, might be linked to disturbances in PPi metabolism. The concentrations of pyrophosphate would be expected to be regulated by hydrolytic enzymes. Studies of the rare inherited disorder, hypophosphatasia, in which lack of alkaline phosphatase is associated with mineralization defects, showed that PPi levels were elevated in both plasma and urine,[76,77] and verified that alkaline phosphatase was the key extracellular enzyme that hydrolyzes pyrophosphate.

Attempts to exploit these concepts by using pyrophosphate and polyphosphates to inhibit ectopic calcification in blood vessels, skin, and kidneys in laboratory animals were successful only when the compounds were injected. Orally administered pyrophosphate and polyphosphates were inactive, due to the hydrolysis of pyrophosphate in the gastrointestinal tract, probably by mucosal brush border phosphatases.[33] During the search for more stable analogues of pyrophosphate that might also have the antimineralization properties of pyrophosphate but that would be resistant to hydrolysis, several different chemical classes were studied. The bisphosphonates (at that time called diphosphonates) were among those studied.

Like pyrophosphate, bisphosphonates had high affinity for bone mineral and were found to prevent calcification both in vitro and in vivo, but, unlike pyrophosphate, were also able to prevent pathological calcification when given orally to rats in vivo.[33] This property of being active by mouth was key to their future use in humans.

Perhaps the most important step towards the future use of bisphosphonates occurred when we found that bisphosphonates also had the novel property of being able to inhibit the dissolution of hydroxyapatite crystals.[32,78] This led to studies to determine whether they might also inhibit bone resorption.[27,32,78] Many studies using a variety of experimental systems showed that they were able to inhibit osteoclast-mediated bone resorption, both in organ cultures of bone in vitro, and in various animal models, e.g., thyroparathyroidectomized rats treated with parathyroid hormone to stimulate bone resorption in vivo.[86,87]

Clinical Applications

Exploration of bisphosphonates as inhibitors of calcification showed some promise, and early applications of etidronate included use in myositis ossificans and in patients who had undergone total hip replacement surgery to prevent subsequent heterotopic ossification and to improve mobility.[10,27] It should be emphasized that these effects required very high doses of etidronate, and that inhibition of skeletal mineralization is not a significant clinical problem when etidronate is used at the low doses recommended in the treatment of osteoporosis.

Another of the early clinical uses of bisphosphonates was as agents for bone imaging, "bone scanning," for which they remain outstandingly useful for detecting bone metastases and other bone lesions. The application of pyrophosphate and simple bisphosphonates as bone scanning agents depends on their strong affinity for bone mineral and their ability to be linked to a gamma-emitting technetium isotope.[35,36]

The most impressive clinical application of bisphosphonates was as inhibitors of bone resorption, often for diseases where no effective treatment existed previously. Thus, bisphosphonates became the treatment of choice for a variety of bone diseases in which excessive osteoclast activity is an important pathological feature, including Paget's disease of bone, metastatic and osteolytic bone disease, and hypercalcemia of malignancy.[9,10,18–20,27,55,56,63,65,80,94]

In the treatment of bone problems associated with malignancy, the skeletal complications of malignancy are reduced with BP therapy, but there is also the important possibility that the survival of patients may be prolonged.[9,55]

More recently, several bisphosphonates, notably etidronate and alendronate, have become established as effective treatments for postmenopausal and other forms of osteoporosis.[1,8,27,42,49,81,95,98,101]

Bisphosphonates in Osteoporosis

In recent years, there has been a remarkably greater awareness of osteoporosis as a major health problem. Alongside the impressive advances in understanding the epidemiology and pathogenesis of osteoporosis and its associated fractures, and in the application of physical and biochemical methods to its diagnosis and evaluation, there have been significant advances in the therapeutic approaches to prevention and treatment of postmenopausal and other forms of osteoporosis.

Bisphosphonates are now well established as successful antiresorptive agents for the prevention and treatment of osteoporosis. In particular, etidronate and alendronate are approved as therapies in many countries, and both can increase bone mass and approximately half fracture rates at the spine, hip, and other sites in postmenopausal women.[8,49,95,97,101] The reduction in fractures may be related not only to the increase in bone mass arising from the inhibition of bone resorption and reduced activation frequency of bone remodeling units, but also to enhanced osteon mineralization.[14,40]

Among the new bisphosphonates, risedronate and ibandronate are coming towards the end of Phase 3 evaluation. In addition to formulations to be taken by mouth, new routes of administration are being studied, especially periodic (e.g., 3 monthly) injections with ibandronate. This has the great attraction of delivering a defined dose without the variability associated with oral administration as well as avoiding potential gastrointestinal intolerance. If this approach is accompanied by greater compliance and convenience, it may become a popular method of treatment.

The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption, but highly selective localisation and retention in bone. Significant side effects of bisphosphonates are minimal.[2,3,17,99] Although there are more similarities than differences between individual compounds and each bisphosphonate is capable of treating any disorder of bone resorption, in practice, different compounds have come to be favored for the treatment of different diseases. To a major extent, the diseases in

which they are used reflect the history of their clinical development and the degree of sponsorship of the relevant clinical trials. Thus, there are several bisphosphonates, including etidronate, clodronate, tiludronate, pamidronate, alendronate, risedronate, and ibandronate, that have been registered for various clinical applications in various countries. Those most used in cancer are pamidronate given parenterally, or clodronate given orally, whereas in osteoporosis the major current drugs are etidronate and alendronate (**Tables 1** and **2**).

Other clinical issues under consideration with bisphosphonates include the choice of therapeutic regimen, e.g., the use of intermittent dosing rather than continuous, intravenous vs. oral therapy, the optimal duration of therapy, the combination with other drugs such as oestrogens, and their extended use in related indications, e.g., glucocorticosteroid-associated osteoporosis, male osteoporosis, childhood osteopenic disorders, arthritis, and other disorders. There is, therefore, much that needs to be done to improve the way in which existing drugs can be used, as well as introducing new ones.

The Relationship Between the Chemical Structure of Bisphosphonates and Their Biological Activity

Bisphosphonates differ from pyrophosphate in that a carbon rather than an oxygen atom bridges the two phosphate residues, which renders bisphosphonates chemically stable and able to withstand incubation in acids or with hydrolytic enzymes (**Figure 1**). The P-C-P moiety is responsible for the strong affinity of the bisphosphonates for the skeleton and allows for a number of variations in structure based on substitution in the R_1 and R_2 positions on the carbon atom (**Figure 2**). The ability of the bisphosphonates to bind to bone mineral, preventing both crystal growth and dissolution, is enhanced when the R_1 side chain (attached to the geminal carbon atom of the P-C-P group) is a hydroxyl group (as in etidronate).[22] The presence of a hydroxyl group at the R_1 position increases the affinity for bone mineral even further, owing to the ability of these bisphosphonates to chelate calcium ions more effectively by tridentate rather than bidentate binding.[22] Bisphosphonates appear to prevent calcification by a physicochemical mechanism, acting as crystal poisons after adsorption to bone surfaces.

The ability of bisphosphonates to inhibit bone resorption in vitro and in vivo requires the P-C-P structure and cannot be achieved with monophosphates, e.g., pentane monophosphonate, or with P-C-C-P or P-N-P compounds.[78] Furthermore, the antiresorptive effect cannot be accounted for simply by adsorption of bisphosphonates to bone mineral and prevention of hydroxyapatite dissolution, since clodronate, for example, although having less affinity for hydroxyapatite than etidronate, is a more potent antiresorptive agent.[78] It is now clear that bisphosphonates inhibit bone resorption by cellular effects on bone-resorbing osteoclasts, rather than by purely physicochemical mechanisms.

Following the successful clinical use of clodronate and etidronate in the 1970s and 1980s, more potent antiresorptive bisphosphonates were studied, which had different R_2 side chains, but in which R_1 (and hence affinity for bone mineral) was unaltered. In particular, bisphosphonates containing a basic primary nitrogen atom in an alkyl chain (as in pamidronate and alendronate) were found to be 10–100-fold more potent than etidronate and clodronate. After this, there was a phase in which synthesis of novel compounds took place specifically to determine their possible effects on calcium metabolism, with the result that compounds highly effective as inhibitors of bone resorption were identified and studied.

These compounds, such as those that contain a tertiary nitrogen (such as ibandronate and olpadronate), are even more potent at inhibiting bone resorption. Among this new generation of compounds that were synthesized to optimize their antiresorptive effects, the most potent antiresorptive bisphosphonates were those containing a nitrogen atom within a heterocyclic ring (as in risedronate and zoledronate), which are up to 10,000-fold more potent than etidronate in some experimental systems.

For maximal potency, the nitrogen atom in the R_2 side chain must be a critical distance away from the P-C-P group, and in a specific spatial configuration. The detailed analysis of structure-activity relationships has allowed the spatial features of the active pharmacophore to be defined with considerable precision, and this can be used in the ongoing chemical design of new and more active compounds. Many hundreds of bisphosphonates have now been synthesized, and more than a dozen have been used in humans.

Although the structure of the R_2 side chain is the major determinant of antiresorptive potency, both phosphonate groups are also required for the drugs to be pharmacologically active. Alterations to one or both phosphonate groups reduces the affinity for bone mineral, and this may be one reason why such BP analogues are less active. For example, replacement of one of the phosphonate hydroxyl groups with a methyl group (to form a phosphonophosphinate) markedly reduces both bone affinity and antiresorptive potency. Methylation of both phosphonate groups in this way (to form a bisphosphinate) leads to loss of bone affinity and loss of antiresorptive activity in vivo.[41] However, BP analogues (for example, a phosphonophosphinate and a phosphonocarboxylate) with similar affinity for bone can have very different antiresorptive potencies.[21] This suggests that the two phosphonate groups (or alternatively, the combination of a phosphonate and a carboxylate group) are required both for targeting to bone and for the molecular mechanism of antiresorptive action, presumably since bisphosphonates mimic naturally occurring, pyrophosphate-containing, compounds.

These studies of the relationships between BP structure and antiresorptive potency suggest that the ability of bisphosphonates to inhibit bone resorption is dependent on two separate properties of the BP molecule.[97] The two phosphonate groups, together with a hydroxyl group at the R_1 position, impart high affinity for bone mineral and act as a "bone hook," allowing rapid and efficient targeting of bisphosphonates to bone mineral surfaces. Once localized within bone, the structure and three-dimensional conformation of the R_2 side chain (as well as the phosphonate groups in the molecule) determine the biological activity of the molecule and influence the ability of the drugs to interact with specific molecular targets. Our understanding of the molecular basis for these differences in potency has become much clearer as a result of recent work.

Mechanisms of Action

Bisphosphonates probably inhibit bone resorption by being selectively taken up and adsorbed to mineral surfaces in bone, whence they are internalized by osteoclasts. Bisphosphonates affect osteoclast-mediated bone resorption in a variety of ways, which include effects on osteoclast recruitment, differentiation, and resorptive activity.[44,45,57,82,84] Once internalized within osteoclasts, bisphosphonates perturb cellular metabolism and induce apoptosis (**Figure 3**). Given the structural similarities to pyrophosphate, it is likely that bisphosphonates internalized by osteoclasts interfere with one or more of the many biochemical processes that involve pyrophosphate-containing compounds. Recent mechanistic studies show that bisphosphonates can be classified into at least two groups with different modes of action.[73] Bisphosphonates that most closely resemble pyrophosphate (such as clodronate and etidronate) can be metabolically

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incorporated into nonhydrolysable analogues of ATP. It is likely that intracellular accumulation of these metabolites within the osteoclast inhibits osteoclast function and may cause osteoclast cell death. In contrast, more potent, nitrogen-containing bisphosphonates, such as alendronate and risedronate, interfere with other metabolic reactions, for example, the mevalonate pathway, and may affect cellular activity and cell survival by interfering with protein prenylation and therefore the signaling functions of key regulatory proteins. These mechanisms are discussed in greater detail below.

Direct Effects of Bisphosphonates on Osteoclasts and Induction of Apoptosis

It is likely that bisphosphonates are selectively internalized by osteoclasts rather than other cell types because of their accumulation in bone and the endocytic activity of osteoclasts. The subcellular space beneath the osteoclast is acidified during the process of bone resorption by the action of vacuolar-type proton pumps in the ruffled border of the osteoclast membrane.[96] The acidic pH of this microenvironment causes dissolution of the hydroxyapatite bone mineral, while the breakdown of the extracellular bone matrix is brought about by the action of proteolytic enzymes. Since bisphosphonates adsorb to bone mineral, especially at sites of bone resorption where the mineral is most exposed,[7,54,84] osteoclasts are the cell type in bone most likely to be exposed to the highest concentrations of free, non-mineral-bound bisphosphonate, as a result of the release of the bisphosphonate from bone mineral in the low pH environment beneath osteoclasts. Thus, low concentrations of bisphosphonates in extracellular fluids could give rise to very much higher local concentrations in the osteoclast resorption lacuna after adsorption to bone, followed by release during the resorption process. For example, it has been estimated that pharmacological doses of alendronate that inhibit bone resorption in vivo could give rise to local concentrations as high as 1 mM alendronate in the resorption space beneath an osteoclast.[84] This is much higher than the concentrations of bisphosphonates required to affect osteoclast morphology and cause osteoclast apoptosis in vitro.[45,83]

Because osteoclasts are highly endocytic, bisphosphonate present in the resorption space is likely to be internalized by endocytosis, and thereby affect osteoclasts directly.[25] The uptake of bisphosphonates by osteoclasts in vivo has been confirmed using radiolabeled alendronate, which was internalized into intracellular vacuoles, and other subcellular compartments such as the cytoplasm, mitochondria, and nuclei.[54,84] After cellular uptake, a characteristic morphological feature of bisphosphonate-treated osteoclasts is the lack of a ruffled border,[83,87] the region of invaginated plasma membrane facing the resorption cavity. Bisphosphonates also disrupt the osteoclast cytoskeleton. Several studies have demonstrated that alendronate and tiludronate disrupt the formation of actin rings in polarised, resorbing osteoclasts.[57,84] This effect appeared to be dependent on cellular uptake (supporting the concept of an intracellular mechanism), since osteoclasts lacking ruffled borders (and hence unable to resorb bone and internalize the released BP) were not affected.[57] Disruption of the cytoskeleton could be brought about indirectly by inhibition of protein kinases or phosphatases that regulate cytoskeletal structure. Indeed, there have been several recent reports that alendronate can inhibit several protein tyrosine phosphatases, without affecting serine or threonine phosphatases.[23,60,88] Tiludronate may also inhibit protein tyrosine phosphatases.[58] A more likely mechanism by which the cytoskeleton may be affected, however, involves loss of function of small GTPases such as Rho and Rac (see below). Nevertheless, inhibitory effects on other enzymes, for example, direct or indirect

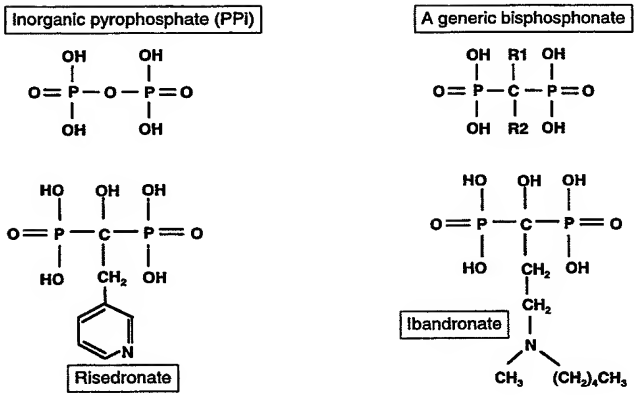

Figure 1. Chemical structures of bisphosphonates (bisphosphonates). Note the relationship to inorganic pyrophosphate, an important intracellular product of metabolism, with similar effects to bisphosphonates on hydroxyapatite crystal behaviour. The two bisphosphonates shown here, risedronate and ibandronate, are close to possible registration based on large phase 3 trials for osteoporosis.

inhibition of the osteoclast proton pumping H^+ATPase,[13,16,106] phosphatases, and lysosomal enzymes,[24,48] could also contribute to the loss of resorptive capacity of osteoclasts after exposure to certain bisphosphonates.

Despite reports that some bisphosphonates do not have toxic effects on osteoclasts,[46] it is clear from many studies that bisphosphonates can reduce osteoclast number[71] and can induce apoptotic cell death in osteoclasts, as first shown by Hughes et al.[45] Apoptosis is a form of cell death that can be distinguished from necrosis on the basis of characteristic changes in morphology (cell and nuclear condensation, chromatin condensation, and nuclear fragmentation) as well as a complex sequence of biochemical events, including activation of proteolytic caspases and internucleosomal DNA cleavage after the activation of an endonuclease. The characteristic morphological features of apoptosis have been described in isolated murine osteoclasts in vitro and in osteoclasts in histological sections from bisphosphonate-treated mice. In addition, DNA cleavage could also be demonstrated in apoptotic osteoclasts in tissue sections by using the TUNEL assay. Selander et al. also found that apoptotic cell death can be induced in isolated osteoclasts by clodronate.[90] Apoptosis triggered by bisphosphonates is not restricted to osteoclasts, however, since macrophages (such as the murine cell line J774)[15,69] and human myeloma cell lines[91,92] also undergo apoptosis after

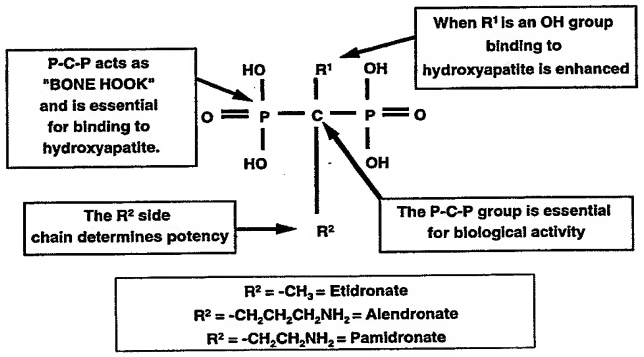

Figure 2. Structure of a bone-active bisphosphonate to show functional domains.

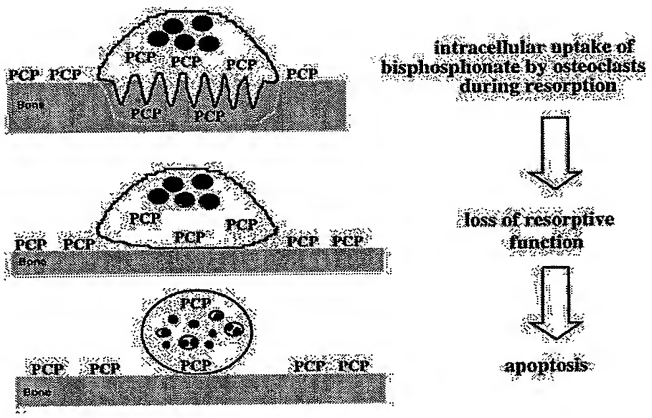

Figure 3. The route by which bisphosphonates affect bone-resorbing osteoclasts.

treatment with several nitrogen-containing bisphosphonates in vitro.

It is still unclear whether bisphosphonate-induced apoptosis is critical to the inhibitory effect of bisphosphonates on bone resorption. Although it is possible that bisphosphonates inhibit bone resorption by causing osteoclast apoptosis, there is currently insufficient evidence to conclude that the inhibitory effect of bisphosphonates on bone resorption can be accounted for solely by an increase in apoptosis. It is perhaps more likely that bisphosphonates inhibit metabolic pathways that firstly affect osteoclast function (e.g., via disruption of the osteoclast cytoskeleton and ruffled border) and cause osteoclast cell death as a later effect. Differences probably also exist between the ability of different bisphosphonates to cause apoptosis, depending on their molecular mechanism of action (see below). Characterization of the events leading to apoptosis in osteoclast surrogates such as J774 macrophages[15,51,52,89] has provided useful insights into the molecular mechanisms of action of bisphosphonates.

Effects of Bisphosphonates on Osteoclast Formation

Since mature, multinucleated osteoclasts are formed by the fusion of mononuclear precursors of hematopoietic origin, bisphosphonates could also inhibit bone resorption by preventing osteoclast formation, in addition to affecting mature osteoclasts. In vitro, bisphosphonates can inhibit dose-dependently the formation of osteoclast-like cells in long-term cultures of human bone marrow.[44] In organ culture also, some bisphosphonates can inhibit the generation of mature osteoclasts, possibly by preventing the fusion of osteoclast precursors.[11,50,62]

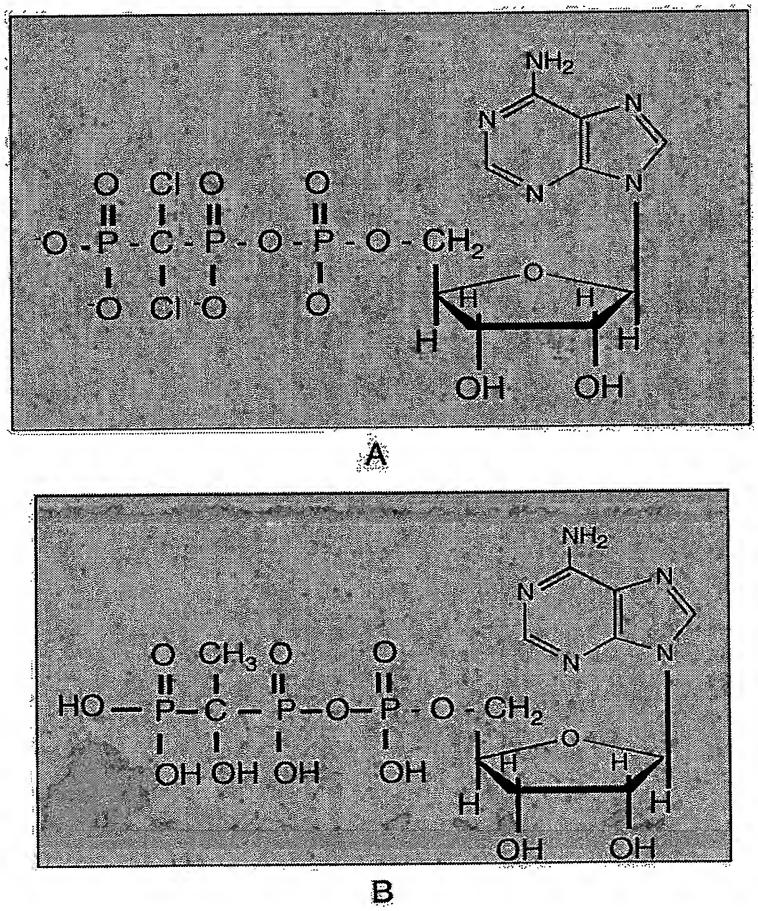

Figure 4. Bisphosphonates can be divided into two classes (those that do or do not contain nitrogen in the R_2 side chain) according to their intracellular actions. This illustrates the metabolites produced by the incorporation of clodronate (A) and etidronate (B) into analogues of ATP (for mechanisms see text).

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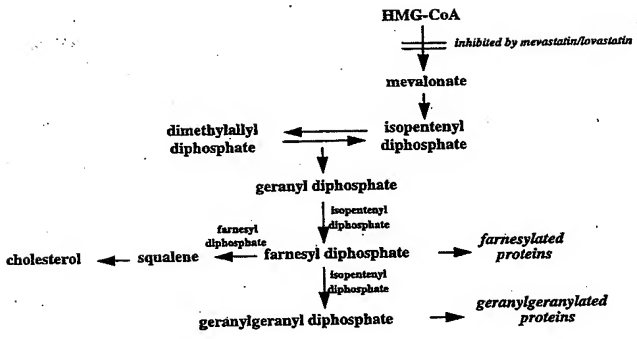

Figure 5. Schematic representation of the mevalonate pathway, leading to synthesis of cholesterol, farnesyl diphosphate, and geranylgeranyl diphosphate. The latter are utilized as substrates for protein prenylation.

There are also reports indicating that bisphosphonates may act via osteoblasts to inhibit bone resorption, by stimulating osteoblasts to produce an osteoclast-inhibitory factor.[59,82,102] Such a factor, of low molecular weight (<10 kD), appears to be present in the conditioned medium of BP-treated osteoblasts.[100] The biochemical structure of this factor remains to be determined. Although effects of bisphosphonates on osteoclast formation and on osteoblasts in vitro have been clearly documented, and, interestingly, in the latter case can occur at nanomolar rather than micromolar concentrations of bisphosphonates, the relevance of these effects in vivo remains uncertain.

The Molecular Mechanisms of Action of Bisphosphonates: Current Opinions

Conversion to bisphosphonate metabolites. As a result of recent work, we have proposed that there two major but distinct molecular mechanisms by which bisphosphonates affect osteoclasts. In studies of bisphosphonates on *Dictyostelium* slime mold amoebae, the growth of which is inhibited by bisphosphonates[72,74] we found that some, but not all, bisphosphonates could be metabolically incorporated by the amoebae into analogues of adenosine triphosphate (ATP or Appp).[70,71] The resulting metabolites contained the P-C-P moiety in place of the β,γ-phosphate groups of ATP, thus resulting in nonhydrolysable (AppCp) nucleotides (**Figure 4**).

Table 1. Major current and potential future uses of bisphosphonates

- Bone scanning agents (linked to technetium-99m)
- Inhibition of calcification
  - Heterotopic bone formation (etidronate at high doses)
  - Dental calculus
- Reducing bone resorption
  - Paget's disease
  - Hypercalcemia of malignancy
  - Multiple myeloma
  - Bone metastases, especially breast cancer
  - Osteoporosis; treatment of established postmenopausal osteoporosis
  - Osteoporosis; prevention of postmenopausal bone loss
  - Glucocorticosteroid-induced bone loss
- Newer and potential clinical indications
  - Extended use in specific indications, e.g., osteoporosis in men
  - Use in children with osteogenesis imperfecta and other osteopenic disorders
  - Use after cardiac or liver transplantation
  - Wider use to prevent glucocorticosteroid induced bone loss in children and adult of both genders and with a spectrum of underlying diseases
  - Extended use in cancers to optimise antitumor effects and survival
  - Prevention of bone loss and erosions in rheumatoid arthritis
  - Possible applications in other joint diseases, such as osteoarthritis
  - Reduction of bone loss associated with periodontal disease
  - Prevention of loosening of joint prostheses

The bisphosphonates that were metabolised by *Dictyostelium* all contain short R_1 and R_2 side chains, with the exception of tiludronate, and are all of relatively low antiresorptive potency. In further studies with cell-free lysates from mammalian cells (which can also incorporate the same bisphosphonates into AppCp nucleotides), we found that the incorporation of bisphosphonates into nucleotide analogues is brought about by members of the family of aminoacyl-tRNA synthetases.[68] These enzymes catalyse a reversible reaction in which an amino acid condenses with ATP to form an aminoacyladenylate, together with the release of pyrophosphate (PPi) (reaction i, shown below). Since this reaction is reversible, it appears that bisphosphonates with short R_1 and R_2 side chains (which most resemble pyrophosphate in structure) can replace PPi in the back reaction (reaction ii). This results in the condensation of a BP (pCp) with an aminoacyladenylate (amino acid-AMP), to form an analogue of ATP (AppCp).

1. Enzyme + amino acid + ATP ⇔ amino-acyl-AMP + PPi
2. Amino-acyl-AMP + pCp ⇒ amino acid + AppCp

The aminoacyl-tRNA synthetases that can utilize a BP in place of pyrophosphate all belong to the Type II subclass of enzymes (e.g., Asn-, Asp-, Gly-, His-, Lys-, Phe-, Ser-aminoacyl-tRNA synthetases) which differ from the Type I subclass in the structure of the catalytic site.[68] Thus, it appears that bisphosphonates with short side chains, but also rather surprisingly tiludronate, can replace pyrophosphate and be accommodated into the active site of Type II aminoacyl-tRNA synthetases. In contrast, the more potent bisphosphonates that contain a nitrogen in the R_2 side chain are not metabolized, presumably since the different and in some cases bulkier structure of the R_2 side chain prevents these bisphosphonates from binding at the active site of the aminoacyl-tRNA synthetase enzymes.

Although the formation of AppCp-type bisphosphonate metabolites was first demonstrated in slime mold amoebae and with cell-free lysates,[64,70,71] we have recently confirmed that intact mammalian cells in vitro (J774 macrophage-like cells and MG63 osteosarcoma cells) can also metabolize clodronate to an analogue of ATP (AppCCl$_2$p).[39] The identity of this metabolite has been confirmed by mass spectrometric analysis of cell lysates from clodronate-treated cells.[6] This technique is highly sensitive and has allowed us to confirm that etidronate and tiludronate can also be metabolized by mammalian cells. These observations raise the likelihood that osteoclasts could also metabolize these bisphosphonates, since in vivo, these are the cells most likely to be exposed to relatively high intracellular concentrations of bisphosphonate. Indeed, in a preliminary study, we have recently used high performance liquid chromatography (HPLC) tandem mass spectrometry to confirm unequivocally that clodronate can be metabolised to AppCCl$_2$p by purified rabbit osteoclasts in vitro.[38]

Since the aminoacyl-tRNA synthetases are cytoplasmic enzymes, the metabolism of bisphosphonates is dependent on cellular uptake.[75] Thus, the AppCp metabolites accumulate in the cell cytoplasm. Owing to the nonhydrolysable nature of the ATP analogues, their accumulation is likely to inhibit numerous intracellular metabolic enzymes, thus having adverse effects on cell function and survival. To test this hypothesis, we performed experiments whereby J774 macrophage-like cells were loaded

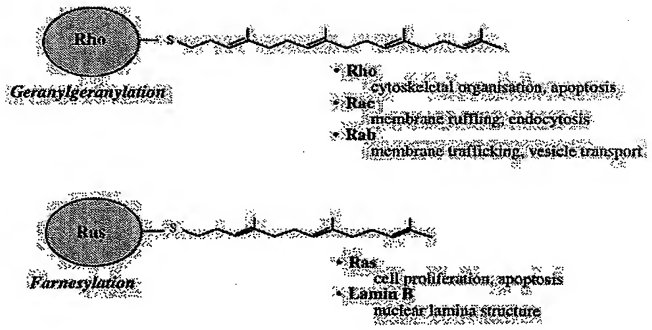

Figure 6. Protein prenylation involves the transfer of a farnesyl or geranylgeranyl group onto the COOH-terminus of small GTPases such as Rho, Rac, Rab, and Ras. The functions of several prenylated proteins are shown.

with the chemically synthesised metabolite of clodronate ($AppCCl_2p$) by using liposome-encapsulated $AppCCl_2p$. This is internalized by the cells by phagocytosis, then $AppCCl_2p$ is released into the cytoplasm after intracellular breakdown of the liposomes. Under these conditions, $AppCC1_2p$ was of similar potency at reducing cell viability as clodronate itself and caused similar changes in morphology to those in clodronate-treated cells.[39] This confirms that AppCp-type BP metabolites are cytotoxic and suggests that some bisphosphonates act as prodrugs, being converted to active metabolites after intracellular uptake by osteoclasts in vivo. However, it remains to be shown exactly how these AppCp-type analogues interfere with cell metabolism.

Inhibition of mevalonate metabolism and of protein prenylation. Potent, nitrogen-containing bisphosphonates (such as alendronate and ibandronate) are not metabolized,[39,60,78] and therefore appear to have a different mechanism of action from that of the bisphosphonates that can be metabolized. Nitrogen-containing bisphosphonates are inhibitors of the mevalonate pathway,[4,5,52] a biosynthetic pathway responsible for the production of cholesterol and isoprenoid lipids, such as isopentenyldiphosphate (IPP), farnesyldiphosphate (FPP), and geranylgeranyldiphosphate (GGPP) (**Figure 5**). FPP and GGPP are required for the posttranslational modification (prenylation) of small GTPases, such as Ras, Rho, and Rac, which are prenylated at a cysteine residue in characteristic COOH-terminal motifs.[104] Small GTPases are important signaling proteins that regulate a variety of cell processes important for osteoclast function, including cell morphology, cytoskeletal arrangement, membrane ruffling, trafficking of vesicles, and apoptosis.[66,67,103,105] Prenylation is required for the correct function of these proteins, since the lipid prenyl group serves to anchor the proteins in cell membranes and may also participate in protein-protein interactions.[53,104] We have recently proposed that the nitrogen-containing bisphosphonates inhibit osteoclastic bone resorption by inhibition of the mevalonate pathway (and hence inhibition of FPP and GGPP synthesis).[52] Hence, indirectly, these bisphosphonates prevent prenylation of proteins, including Ras, and cause loss of function of these small GTPases.[52]

Several lines of evidence indicate that the mevalonate pathway is essential for osteoclast function and is the route by which the nitrogen-containing bisphosphonates affect osteoclasts. First, our studies with J774 macrophages (which undergo BP-induced apoptosis, like osteoclasts) demonstrated that nitrogen-containing bisphosphonates, but not clodronate, inhibit the incorporation of [^{14}C]mevalonate into prenylated proteins, thus demonstrating that nitrogen-containing bisphosphonates inhibit enzymes in the mevalonate pathway.[52] Furthermore, changes to the structure of the nitrogen-containing R_2 side chain or to the phosphonate groups that influence antiresorptive potency also influence in a similar manner the ability to inhibit protein prenylation.[51] Hence, potent antiresorptive bisphosphonates are effective inhibitors of protein prenylation, whereas less potent antiresorptive bisphosphonates are less effective inhibitors of protein prenylation. This was the first time that the structure-activity relationships of bisphosphonates could be correlated with a specific biochemical effect. Importantly, we also found that addition of intermediates of the mevalonate pathway (such as FPP and GGPP) could prevent BP-induced apoptosis in J774 macrophages.[51] In studies with organ cultures of calvariae, mevalonate could also reverse BP-induced inhibition of osteoclast formation and bone resorption.[52] This was clear evidence that the dominant mechanism of action of some bisphosphonates involves inhibition of the mevalonate pathway, rather than other mechanisms such as inhibition of protein tyrosine phosphatases.[23,60,88] Finally, to confirm that inhibition of the mevalonate pathway could account for the antiresorptive effects of bisphosphonates, we examined the effect of mevastatin, another inhibitor (unrelated to bisphosphonates) of the mevalonate pathway. Mevastatin (an inhibitor of HMG-CoA reductase) was found to be even more potent than bisphosphonates at inhibiting bone resorption in vitro and, like bisphosphonates, caused apoptosis of osteoclasts and J774 macrophages in vitro.[53] In addition, several characteristic features of BP-induced apoptosis in J774 cells (e.g., the time of occurrence of apoptosis, and the dependence of caspase activation and apoptosis on protein synthesis) were strikingly similar to the features of

Table 2. List of bisphosphonates used in clinical studies and under clinical development

Bisphosphonate	R1	R2	Main current uses
Etidronate[a]	OH	CH_3	Osteoporosis, Paget's disease
Clodronate[a]	CI	CI	Metastases, myeloma
Pamidronate[a]	OH	CH_2CH_2 NH_2	Hypercalcaemia, myeloma, Paget's disease
Alendronate[a]	OH	$(CH_2)_3$ NH_2	Osteoporosis and other indications
Risedronate[a]	OH	CH_2-3-pyridine	Registration pending for osteoporosis
Tiludronate[a]	H	CH_2-S-phenyl-CI	Paget's disease
Ibandronate[a]	OH	CH_2CH_2 $N(CH_3)$ (pentyl)	In development, osteoporosis and several diseases
Zoledronate	OH	CH_2-imidazole	In development, several diseases
YH529	OH	CH_2-2-imidazo-pyridinyl	
Incadronate (YM175)	H	N-(cyclo-heptyl)	
Olpadronate	OH	CH_2CH_2 $N(CH_3)_2$	
Neridronate	OH	$(CH_2)_5$ NH_2	
EB-1053	OH	CH_2-1-pyrrolidinyl	

[a]Indicates bisphosphonates already approved for one or more indications in one or more countries.

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mevastatin-induced apoptosis,[15] supporting the notion that bisphosphonates cause apoptosis by preventing protein prenylation.

More recently, Fisher et al.[25] have shown that lovastatin inhibits osteoclast formation in cocultures of osteoblasts and murine bone marrow, and inhibits bone resorption by isolated osteoclasts in vitro. This latter study also showed that the effects of alendronate and lovastatin could be overcome by the addition of geranylgeraniol (which can be used for protein geranylgeranylation) but not farnesol (which is utilized for protein farnesylation). Hence, it appears that, although nitrogen-containing bisphosphonates can prevent both farnesylation and geranylgeranylation of proteins (probably by inhibiting enzymes required for synthesis of FPP and GGPP), loss of geranylgeranylated proteins in osteoclasts is of greater consequence than loss of farnesylated proteins. This is consistent with the known role of geranylgeranylated proteins such as Rho, Rac, and Rab in processes that are fundamental to osteoclast formation and function (e.g., cytoskeletal rearrangement, membrane ruffling, and vesicular trafficking.[43,103] Furthermore, unlike with alendronate, the effect of clodronate on osteoclast formation and bone resorption in calvariae in vitro could not be overcome by supplementation with mevalonate,[25] reaffirming the hypothesis that clodronate and alendronate have different molecular mechanisms of action.

The exact enzymes of the mevalonate pathway that are inhibited by bisphosphonates have not yet been fully identified. However, incadronate and ibandronate are known to be inhibitors of squalene synthase, an enzyme in the mevalonate pathway required for cholesterol biosynthesis.[4,5] Alendronate and pamidronate are less potent inhibitors of squalene synthase but can also inhibit sterol biosynthesis, suggesting that these bisphosphonates may inhibit upstream enzymes of the mevalonate pathway other than squalene synthase.[4] Several enzymes of the pathway utilize an isoprenoid diphosphate as a substrate (IPP isomerase, FPP synthase, GGPP synthase, squalene synthase) and thus are likely to have similar substrate binding sites. Thus, if nitrogen-containing bisphosphonates act as substrate analogues of an isoprenoid diphosphate, it is likely that these bisphosphonates actually inhibit several enzymes of the mevalonate pathway. This could explain why alendronate and pamidronate can inhibit sterol biosynthesis without being potent inhibitors of squalene synthase.[4] Thus, the overall antiresorptive potency of the nitrogen-containing bisphosphonates may ultimately depend on the number of enzymes that are inhibited, or the combination of enzymes that are inhibited. Further studies in this area will surely lead to the identification of the exact molecular targets of bisphosphonates and clarification of the structure-activity relationships of these compounds.

Taken together, these observations clearly indicate that bisphosphonates can be grouped into two classes; those that can be metabolized into nonhydrolysable analogues of ATP (the least potent bisphosphonates), and those that are not metabolized but that can inhibit protein prenylation (the potent, nitrogen-containing bisphosphonates). The identification of two such classes may help to explain some of the other pharmacologic differences between the two classes, for example, the ability of the nitrogen-containing bisphosphonates to cause an acute phase response in vivo.[2,3,85,89]

Future Prospects

It has taken over 30 years since the discovery of the profound effects of the bisphosphonates on calcium metabolism for them to become well established as clinically successful antiresorptive agents, and their availability has enabled new approaches to the therapy of bone diseases.

There have now been many years of mostly favorable experience with the use of bisphosphonates in diseases such as Paget's disease of bone, myeloma, and bone metastases. Bisphosphonates represent an important class of drugs for the treatment of these bone diseases, and their real potential, particularly in managing tumor-associated bone disease, has yet to be fully realized.

Their application in osteoporosis is relatively recent and was spurred on by the development of techniques to measure bone mass with precision, the increased awareness of osteoporosis as a major socio-economic problem, and the willingness of the larger pharmaceutical companies to invest in clinical studies on the scale necessary to demonstrate effects on fractures.

The difficulties of bringing these drugs to the market is illustrated by those that fall by the wayside, such as oral pamidronate and tiludronate. There are important lessons to be learned from the need to do good dose-response studies during phase 2 development and making appropriate choices of doses.

However, despite the enormous potential for developing "better" bisphosphonates based on current knowledge of their structure-activity properties, it is unlikely, given the high cost of development, that further agents will be developed unless they offer distinct advantages over currently available bisphosphonates. For example, attempts to improve intestinal absorption, e.g., by better formulations or by creating prodrugs, such as peptide derivatives, have not so far resulted in clinically significant successes.

The recent elucidation of the likely mode of action of bisphosphonates within cells opens up the possibility of utilizing subtle and potentially important differences between classes of bisphosphonates and individual compounds. Their potential antitumor effects and apparent ability to prolong survival in patients with myeloma or breast cancer metastases merit further study. Other clinical indications ripe for future study include the prevention of bone loss and erosions in rheumatoid arthritis, possible applications in other joint diseases, and the reduction of bone loss associated with periodontal disease, and loosening of joint prostheses.

References

1. Adachi, J. D., Bensen, W. G., Brown, J., et al. Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. N Engl J Med 337:381–387; 1997.
2. Adami, S., Bhalla, A. K., Dorizzi, R., et al. The acute-phase response after bisphosphonate administration. Calcif Tissue Int 41:326–331; 1987.
3. Adami, S., and Zamberlan, N. Adverse effects of bisphosphonates. Drug Safety 14:158–170; 1996.
4. Amin, D., Cornell, S. A., Gustafson, S. K., et al. Bisphosphonates used for the treatment of bone disorders inhibit squalene synthase and cholesterol biosynthesis. J Lipid Res 33:1657–1663; 1992.
5. Amin, D., Cornell, S. A., Perrone, M. H., and Bilder, G. E. 1-hydroxy-3-(methylpentylamino)-propylidene-1,1-bisphosphonic acid as a potent inhibitor of squalene synthase. Drug Res 46:759–762; 1996.
6. Auriola, S., Frith, J., Rogers, M. J., Koivuniemi, A., and Mönkkönen, J. Identification of adenine nucleotide-containing metabolites of bisphosphonate drugs using ion-pair liquid chromatography-electrospray mass spectrometry. J Chrom B 704:187–195; 1997.
7. Azuma, Y., Sato, H., Oue, Y., et al. Alendronate distributed on bone surfaces inhibits osteoclastic bone resorption in vitro and in experimental hypercalcemia models. Bone 16:235–245; 1995.
8. Black, D. M., Cummings, S. R., Karpf, D. B., et al. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet 348:1535–1541; 1996.
9. Berenson, J. R., Lichtenstein, A., Porter, L., et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. N Engl J Med 334:488–493; 1996.
10. Bijvoet, O., Fleisch, H., Canfield, R. E., and Russell, R. G. G., Eds. Bisphosphonates on Bone. Elsevier Science B. V.: Amsterdam; 1995; paper 1–455.

11. Boonekamp, P. M., van der Wee-Pals, L. J. A., van Wijk-van Lennep, M. L. L., Wil Thesing, C., and Bijvoet, O. L. M. Two modes of action of bisphosphonates on osteoclastic resorption of mineralized matrix. Bone Miner 1:27–39; 1986.
12. Breuil, V., Cosman, F., Stein, L., et al. Human osteoclast formation and activity in vitro: effects of alendronate. J Bone Miner Res 13:1721–1729; 1998.
13. Carano, A., Teitelbaum, S. A., Konsek, J. D., Schlesinger, P. H., and Blair, H. C. Bisphosphonates directly inhibit the bone resorption activity of isolated avian osteoclasts in vitro. J Clin Invest 85:456–461; 1990.
14. Chavassieux, P. M., Arlot, M. E., Reda, C., Wei, L., Yates, A. J., and Meunier, P. J. Histomorphometric assessment of the long term effects of alendronate on bone quality and remodeling in patients with osteoporosis. J Clin Invest 100:1475–1480; 1997.
15. Coxon, F. P., Benford, H. L., Russell, R. G. G., and Rogers, M. J. Protein synthesis is required for caspase activation and induction of apoptosis by bisphosphonate drugs. Mol Pharmacol 54:631–638; 1998.
16. David, P., Nguyen, H., Barbier, A., and Baron, R. The bisphosphonate tiludronate is a potent inhibitor of the osteoclast vacuolar H^+ ATPase. J Bone Miner Res 11:1498–1507; 1996.
17. DeGroen, P. C., Lubbe, D. F., Hirsch, L. J., et al. Esophagitis associated with the use of alendronate. N Engl J Med 355:1016–1021; 1996.
18. Delmas, P. D. and Meunier, P. J. The management of Paget's disease of bone. N Engl J Med 336:558–566; 1997.
19. Diel, I. J., Solomayer, E.-F., Costa, S. D., et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med 339:357–363; 1998.
20. Douglas, D. L., Russell, R. G. G., Preston, C. J., et al. Effect of dichloromethylene diphosphonate in Paget's disease of bone and in hypercalcaemia due to primary hyperparathyroidism or malignant disease. Lancet 1:1043–1047; 1980.
21. Ebetino, F. H., Bayless, A. V., Amburgey, J., Ibbotson, K. J., Dansereau, S., and Ebrahimpour, A. Elucidation of a pharmacore for the bisphosphonate mechanism of bone antiresorptive activity. Phosphorus, Sulfur and Silicon 109/110:217–220; 1996.
22. Ebetino, F. H., Francis, M. D., Rogers, M. J., and Russell, R. G. G. Etidronate. Mechanisms of action of etidronate and other bisphosphonates. Rev Contemp Pharma 9:233–243; 1998.
23. Endo, N., Rutledge, S. J., Opas, E. E., Vogel, R., Rodan, G. A., and Schmidt, A. Human protein tyrosine phosphatases: alternative splicing and inhibition by bisphosphonates. J Bone Miner Res 11:535–543; 1996.
24. Felix, R., Russell, R. G. G., Fleisch, H. The effect of several diphosphonates on acid phosphohydrolases and other lysosomal enzymes. Biochim Biophys Acta 429:429–438; 1976.
25. Fisher, J. E., Rogers, M. J., Halasy, J. M., et al. Mechanism of action of alendronate: geranylgeraniol, an intermediate of the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption and kinase activation in vitro. Proc Natl Acad Sci USA 96:133–138; 1999.
26. Flanagan, A. M., Chambers, T. J. Dichloromethylenebisphosphonate (Cl_2MBP) inhibits bone resorption through injury to osteoclasts that resorb Cl_2MBP-coated bone. Bone Miner 6:33–43; 1986.
27. Fleisch, H. Bisphosphonates in Bone Disease. From the Laboratory to the Patient, Third Edition. New York: The Parthenon Publishing Group; 1997.
28. Fleisch, H. Bisphosphonates: mechanisms of action. Endocr Rev 19:80–100; 1998.
29. Fleisch, H. and Bisaz, S. Isolation from urine of pyrophosphate, a calcification inhibitor. Am J Physiol 203:671–675; 1962.
30. Fleisch, H. and Neuman, W. F. Mechanisms of calcification: role of collagen, polyphosphates, and phosphatase. Am J Physiol 200:1296–1300; 1961.
31. Francis, M. D., Russell, R. G. G., and Fleisch, H. Diphosphonates inhibit formation of calcium phosphate crystals *in vitro* and pathological calcification *in vivo*. Science 165:1264–1266; 1969.
32. Fleisch, H., Russell, R. G. G., and Francis, M. D. Diphosphonates inhibit hydroxyapatite dissolution *in vitro* and bone resorption in tissue culture and *in vivo*. Science 165:1262–1264; 1969.
33. Fleisch, H., Russell, R. G. G., Bisaz, S., Muhlbauer, R. C., and Williams, D. A. The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. Eur J Clin Invest 1:12–18; 1970.
34. Fleisch, H., Russell, R. G. G., and Straumann, F. Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 212:901–903; 1966.
35. Fogelman, I., Bessent, R. G., Turner, J. F., Citrin, D. L., Boyce, I. T., and Greig, W. R. The use of whole-body retention of Tc-99m diphosphonate in the diagnosis of metabolic bone disease. J Nucl Med 19:270–275; 1978.
36. Francis, M. D. and Fogelman, I. 99mTc-diphosphonate uptake mechanisms on bone. In: Fogelman, I., Ed. Bone Scanning in Clinical Practice. London: Springer Verlag; 1987; 1–6.
37. Francis, R. M. Oral bisphosphonates in the treatment of osteoporosis: a review. Curr Ther Res 56:831–851; 1998.
38. Frith, J. C., Coxon, F. P., Auriola, S., et al. The molecular mechanism of action of clodronate in osteoclasts. J Bone Miner Res 23(Suppl.):551; 1998.
39. Frith, J. C., Mönkkönen, J., Blackburn, G. M., Russell, R. G. G., and Rogers, M. J. Clodronate and liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5′-β,γ-dichloromethylene) triphosphate, by mammalian cells *in vitro*. J Bone Miner Res 12:1358–1367; 1996.
40. Garnero, P., Shih, W. J., Gineyts, E., Karpf, D. G., and Delmas, P. D. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. J Clin Endocrinol Metab 79:1693–1700; 1994.
41. Geddes, A. D., D'Souza, S. M., Ebetino, F. H., and Ibbotson, K. J. Bisphosphonates: structure-activity relationships and therapeutic implications. Bone Miner Res 8:265–306; 1994.
42. Glorieux, F. H., Bishop, N. J., Plotkin, H., Chabot, G., Lanoue, G., and Travers, R. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. N Engl J Med 33:947–952; 1998.
43. Hall, A. Rho GTPases and the actin cytoskeleton. Science 279:509–514; 1998.
44. Hughes, D. E., MacDonald, B. R., Russell, R. G. G., and Gowen, M. Inhibition of osteoclast-like cell formation by bisphosphonates in long-term cultures of human bone marrow. J Clin Invest 83:1930–1935; 1989.
45. Hughes, D. E., Wright, K. R., Uy, H. L., et al. Bisphosphonates promote apoptosis in murine osteoclasts *in vitro* and *in vivo*. J Bone Miner Res 10:1478–1487; 1995.
46. Ito, M., Chokki, M., Ogino, Y., et al. Comparison of the cytotoxic effects of bisphosphonates in vitro and in vivo. Calcif Tiss Int 63:143–147; 1998.
47. Johnson, S. and Johnson, F. N., Eds. Etidronate in Osteoporosis. Rev Contemp Pharm 9:1–292; 1998.
48. Lerner, U. H., and Larsson, A. Effects of four bisphosphonates on bone resorption, lysosomal enzyme release, protein synthesis and mitotic activities in mouse calvarial bones in vitro. Bone 8:179–189; 1987.
49. Liberman, U. A., Weiss, S. R., Broll, J., et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 333:1437–1443; 1995.
50. Löwik, C. W. G. M., van der Pluijm, G., van der Wee-Pals, L. J. A., Bloys van Treslong-de Groot, H., and Bijvoet, O. L. M. Migration and phenotypic transformation of osteoclast precursors into mature osteoclasts: the effect of a bisphosphonate. J Bone Miner Res 3:185–192; 1988.
51. Luckman, S. P., Coxon, F. P., Ebetino, F. H., Russell, R. G. G., and Rogers, M. J. Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: evidence from structure-activity relationships in J774 macrophages. J Bone Miner Res 13:1668–1678; 1998.
52. Luckman, S. P., Hughes, D. E., Coxon, F. P., Russell, R. G. G., and Rogers, M. J. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res 13:581–589; 1998.
53. Marshall, C. Protein prenylation: a mediator of protein-protein interactions. Science 259:1865–1866; 1993.
54. Masarachia, P., Weinreb, M., Balena, R., and Rodan, G. A. Comparison of the distribution of 3H-alendronate and 3H-etidronate in rat and mouse bones. Bone 19:281–290; 1996.
55. McCloskey, E. V., MacLennan, I. C. M., Drayson, M., Chapman, C., Dunn, J., and Kanis, J. A. A randomized trial of the effect of clodronate on skeletal morbidity in multiple myeloma. MRC Working Party on Leukaemia in Adults. Br J Haematol 100:317–325; 1998.
56. Mundy, G. R. and Yoneda, T. Bisphosphonates as anticancer drugs. N Engl J Med 339:398–400; 1998.
57. Murakami, H., Takahashi, N., Sasaki, T., et al. A possible mechanism of the specific action of bisphosphonates on osteoclasts: tiludronate preferentially affects polarized osteoclasts having ruffled borders. Bone 17:137–144; 1995.
58. Murakami, H., Takahashi, N., Tanaka, S., et al. Tiludronate inhibits protein tyrosine phosphatase activity in osteoclasts. Bone 20:399–404; 1997.
59. Niskikawa, M., Akatsu, T., Katayama, Y., et al. Bisphosphonates act on osteoblastic cells and inhibit osteoclast formation in mouse marrow cultures. Bone 18:9–14; 1996.
60. Olpas, E. E., Rutledge, S. J., Golub, E., et al. Alendronate inhibition of protein-tyrosine-phosphatase-Meg1. Biochem Pharmacol 54:721–727; 1997.

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61. Papapoulos, S. E. Pharmacodynamics of bisphosphonates in man. In: Bijvoet, O., Fleisch, H. A., Canfield, R. E., Russell, R. G. G., Eds. Bisphosphonates on Bone. Elsevier Science B.V.: Amsterdam; 1995.
62. Papapoulos, S. E., Hoekman, K., Lowik, C. W. G. M., Vermeij, P., and Bijvoet, O. L. M. Application of an in vitro model and a clinical protocol in the assessment of the potency of a new bisphosphonate. J Bone Miner Res 4:775–781; 1988.
63. Paterson, A. D., Kanis, J. A., Cameron, E. C., et al. The use of dichloromethylene diphosphonate for the management of hypercalcaemia in multiple myeloma. Br J Haematol 54:121–131; 1983.
64. Pelorgeas, S., Martin, J.-P., and Satre, M. Cytotoxicity of dichloromethane diphosphonate and of 1-hydroxyethane-1,1-diphosphonate in the amoebae of the slime mould *Dictyostelium discoideum*. Biochem Pharmacol 44:2157–2163; 1992.
65. Ralston, S. H., Gallacher, S. J., Patel, U., Fraser, W. D., Dryburgh, F. J., Boyle, I. T. Comparison of three bisphosphonates in cancer-associated hypercalcaemia. Lancet 2:1180–1182; 1989.
66. Ridley, A. J. and Hall, A. The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 70:389–399; 1992.
67. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. The small GTP-binding protein, rac, regulates growth factor-induced membrane ruffling. Cell 70:401–410; 1992.
68. Rogers, M. J., Brown, R. J., Hodkin, V., Russell, R. G. G., and Watts, D. J. Bisphosphonates are incorporated into adenine nucleotides by human aminoacyl-tRNA synthetase enzymes. Biochem Biophys Res Commun 224:863–869; 1996.
69. Rogers, M. J., Chilton, K. M., Coxon, F., et al. Bisphosphonates induce apoptosis in mouse macrophage-like cells by a nitric-oxide-independent mechanism. J Bone Miner Res 11:1482–1491; 1996.
70. Rogers, M. J., Ji, X., Russell, R. G. G., et al. Incorporation of bisphosphonates into adenine nucleotides by amoebae of the cellular slime mould *Dictyostelium discoideum*. Biochem J 303:303–311; 1994.
71. Rogers, M., Russell, R. G. G., Blackburn, G. M., Williamson, M. P., and Watts, D. J. Metabolism of halogenated bisphosphonates by the cellular slime mould *Dictyostelium discoideum*. Biochem Biophys Res Commun 189:414–423; 1992.
72. Rogers, M. J., Watts, D. J., Russell, R. G. G., et al. Inhibitory effects of bisphosphonates on growth of amoebae of the cellular slime mould *Dictyostelium discoideum*. J Bone Miner Res 9:1029–1039; 1994.
73. Rogers, M. J., Watts, D. J., and Russell, R. G. G. Overview of bisphosphonates. Cancer 80:1652–1669; 1997.
74. Rogers, M. J., Xiong, X., Brown, R. J., et al. Structure-activity relationships of new heterocycle-containing bisphosphonates as inhibitors of bone resorption and as inhibitors of growth of *Dictyostelium discoideum* amoebae. Mol Pharmacol 47:398–402; 1995.
75. Rogers, M. J., Xiong, X., Ji, X., et al. Inhibition of growth of *Dictyostelium discoideum* amoebae by bisphosphonates is dependent on cellular uptake. Pharm Res 14:625–630; 1997.
76. Russell, R. G. G. Excretion of inorganic pyrophosphate in hypophosphatasia. Lancet 2:461–464; 1965.
77. Russell, R. G. G., Bisaz, S., Donath, A., Morgan, D. B., and Fleisch, H. Inorganic pyrophosphate in plasma in normal persons and in patients with hypophosphatasia, osteogenesis imperfecta and other disorders of bone. J Clin Invest 50:961–969; 1971.
78. Russell, R. G. G., Muhlbauer, R. C., Bisaz, S., Williams, D. A., and Fleisch, H. The influence of pyrophosphate, condensed phosphates, phosphonates and other phosphate compounds on the dissolution of hydroxyapatite in vitro and on bone resorption induced by parathyroid hormone in tissue culture and in thyroparathyroidectomised rats. J Calcif Tissue Res 6:83–196; 1970.
79. Russell, R. G. G. and Rogers, M. J. Introduction to bisphosphonates and the clinical pharmacology of alendronate. Br J Rheumatol 36:10–14; 1997.
80. Russell, R. G. G., Smith, R., Preston, C., Walton, R. J., and Woods, C. F. Diphosphonates in Paget's disease. Lancet 1:894–898; 1974.
81. Saag, K. G., Emkey, K., Schnitzer, T. J., et al. Alendronate for the prevention and treatment of glucocorticoid-induced osteoporosis. N Engl J Med 339: 292–299; 1998.
82. Sahni, M., Guenther, H., Fleisch, H., Collin, P. and Martin, T. J. Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. J Clin Invest 91:2004–2011; 1993.
83. Sato, M. and Grasser, W. Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. J Bone Miner Res 5:31–40; 1990.
84. Sato, M., Grasser, W., Endo, N., et al. Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. J Clin Invest 88:2095–2105; 1991.
85. Sauty, A., Pecherstorfer, M., Zimmerroth, I., et al. Interleukin-6 and tumor necrosis factor alpha levels after bisphosphonate treatment *in vitro* and in patients with malignancy. Bone 18:133–139; 1996.
86. Schenk, R., Eggli, P., Fleisch, H., and Rosini, S. Quantitative morphometric evaluation of the inhibitory activity of new aminobisphosphonates on bone resorption in the rat. Calcif Tissue Int 38:342–349; 1986.
87. Schenk, R., Merz, W. A., Muhlbauer, R., Russell, R. G. G., and Fleisch, H. Effect of ethane-1-hydroxy-1,1-diphosphonate (EHDP) and dichloromethylene diphosphonate (Cl_2MDP) on the calcification and resorption of cartilage and bone in the tibial epiphysis and metaphysis of rats. Calcif Tissue Res 11:196–214; 1973.
88. Schmidt, A., Rutledge, S. J., Endo, N., et al. Alendronate inhibition of protein tyrosine phosphatase activity. Proc Natl Acad Sci USA 93:3068–3073; 1995.
89. Schweitzer, D. H., Oostendorp-van de Ruit, M., van der Pluijm, G., Lowik, C. W. G. M., Papapoulos, S. E. Interleukin-6 and the acute phase response during treatment of patients with Paget's disease with the nitrogen-containing bisphosphonate dimethylaminohydroxypropylidene bisphosphonate. J Bone Miner Res 10:956–962; 1995.
90. Selander, K. S., Mönkkönen, J., Karhukorpi, E.-K., Härkönen, P., Hannuniemi, R., and Väänänen, H. K. Characteristics of clodronate-induced apoptosis in osteoclasts and macrophages. Mol Pharmacol 50:1127–1138; 1996.
91. Shipman, C. M., Croucher, P. I., Russell, R. G. G., and Rogers, M. J. The bisphosphonate incadronate (YM175) causes apoptosis of human myeloma cells in vitro by inhibiting the mevalonate pathway. Cancer Res 58:5294–5297; 1998.
92. Shipman, C. M., Rogers, M. J., Apperley, J. F., Russell, R. G. G., and Croucher, P. I. Bisphosphonates induce apoptosis in human myeloma cells; a novel anti-tumour activity. Br J Haematol 98:665–672; 1997.
93. Sietsema, W. K., Ebetino, F. H., Salvagno, A. M., and Bevan, J. A. Antiresorptive dose-response relationships across three generations of bisphosphonates. Drugs Exp Clin Res 15:389–396; 1989.
94. Smith, R., Russell, R. G. G., Bishop, M. Diphosphonate and Paget's disease of bone. Lancet 1:945–947; 1971.
95. Storm, T., Thamsborg, G., Steiniche, T., et al. Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. N Engl J Med 322:1265–1271; 1990.
96. Suda, T., Nakamura, I., Jimi, E., Takahashi, N. Regulation of osteoclast function. J Bone Miner Res 12:869–879; 1997.
97. van Beek, E., Lowik, C., Oue, I., and Papapoulos, S. Dissociation of binding and antiresorptive properties of hydroxybisphosphonates by substitution of the hydroxyl with an amino group. J Bone Miner Res 11:1492–1497; 1996.
98. van Staa, T. P., Abenhain, L., and Cooper, C. Use of cyclical etidronate and prevention of non-vertebral fractures. Br J Rheumatol 37:87–94; 1998.
99. van Staa, T. P. Post marketing survey with ICT-etidronate therapy. Rev Contemp Pharmacol 9:277–286; 1998.
100. Vitte, C., Fleisch, H., and Guenther, H. L. Bisphosphonates induce osteoblasts to secrete an inhibitor of osteoclast-mediated resorption. Endocrinology 137: 2324; 1996.
101. Watts, N. B., Harris, S. T., Genant, H. K., et al. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. N Engl J Med 323:73–79; 1990.
102. Yu, X., Scholler, J., and Foged, N. T. Interaction between effects of parathyroid hormone and bisphosphonate on regulation of osteoclast activity by the osteoblast-like cell line UMR-106. Bone 19:339–345; 1996.
103. Zerial, M. and Stenmark, H. Rab GTPases in vesicular transport. Curr Opin Cell Biol 5:613–620; 1993.
104. Zhang, F. L. and Casey, P. J. Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241–249; 1996.
105. Zhang, D., Udagawa, N., Nakamura, I., et al. The small GTP-binding protein, *Rho* p21, is involved in bone resorption by regulating cytoskeletal organization in osteoclasts. J Cell Sci 108:2285–2292; 1995.
106. Zimolo, Z., Wesolowski, G., and Rodan, G. A. Acid extrusion is induced by osteoclast attachment to bone. J Clin Invest 96:2277–2283; 1995.

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